The *HER2* I655V Polymorphism and Breast Cancer Risk in Ashkenazim

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Background: Over-expression of the human epidermal growth factor receptor 2 (Her2) protooncogene is associated with poor prognosis among female patients with breast cancer. A polymorphism in the HER2 gene (I655V) has been associated with an elevated risk of breast cancer in some ethnic groups.

Methods: Subjects from a community-based study of 5318 Ashkenazim from the Washington, DC area were selected for analysis of the I655V HER2 germline polymorphism. We estimated age-specific breast cancer risk from HER2 I655V based on the family history data, using the female first-degree relatives of the study participants and a novel extension of the kin cohort method.

Results: The estimated cumulative risk of breast cancer to age 70 was approximately 30% higher among HER2 I655V carriers than noncarriers (RR = 1.33; 95% confidence interval [CI] = 1.03–1.83). The effect of the allele seems stronger at younger ages (among women younger than 50 years, RR = 2.11; CI = 1.39–3.28) and especially among younger women with a family history of breast cancer (RR = 8.9; CI = 1.9–19.7). Increased risk of breast cancer associated with the I655V allele was also observed among BRCA1/2 mutation carriers, although these results are based on small numbers.

Conclusion: These analyses suggest that the HER2 valine allele might be associated with increased risk of breast cancer, especially in young women and in women with a family history of the disease.

Key Words: kin-cohort, polymorphism, risk, breast cancer, epidermal growth factor receptor

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Estimates of lifetime breast cancer risk among women who carry a *BRCA1* or *BRCA2* mutation, or are from kindreds with multiple cases of breast and/or ovarian cancer, range from 40-87%.[1-5] Some of this variation might by an artifact of differences in study design or a consequence of allelic heterogeneity (different risks associated with different mutations in the same gene).[6,7] Also, statistical modeling[8,9] suggests that other genetic factors are likely to modify the risk of breast and ovarian cancers. Such genes might contain common, low-penetrance variants that could have a main effect themselves or could modify the effect of the known moderately to highly penetrant genes.

One such candidate gene is the human epidermal growth factor receptor 2 gene (*HER2; EGFR2, ERBB2*), located on chromosome 17q. A homologue of the rat *neu* oncogene, Her2 is a 185-kD transmembrane glycoprotein with tyrosine kinase activity and is the second of 4 human epidermal growth factor (EGF) receptors identified to date. In approximately 30% of human breast tumors, oncogenic activation occurs through Her2/*neu* gene amplification or overexpression,[10-13] and the presence of Her2 is associated with poor clinical prognosis.[10,14-17] In 1991, Papewalis et al.[18] identified a valine to isoleucine polymorphism in the transmembrane region of *HER2* at codon 655. This isoleucine allele is the most common; we refer to it as the I655V polymorphism.

Her2 is a member of the Her family of tyrosine kinase receptors in which binding of growth factors regulates cell growth, proliferation, and differentiation through dimerization and of various Her receptor combinations.[19] Although Her2 has no known ligand, it is generally the preferred heterodimerization partner for the other Her receptors[20] and can be critical for potentiation of the signal.[21] The location of the transmembrane I655V polymorphism corresponds to a similar single point mutation found in *neu*, the rat homolog of Her2 that results in a valine to glutamic acid substitution at position 664 of the transmembrane domain. The V664E mutation mimics ligand induction and renders constitutive Her2 dimerization and activation.[10,12,22] A possible functional role for the I655V *HER2* polymorphism has not been studied. However, there is evidence suggesting that isoleu-

cine to valine changes might alter the hydrophobicity of proteins affecting the conformational stability of the hydrophobic domains[23] such as transmembrane domains.

In 2000, Xie et al.[24] evaluated this polymorphism in a population-based case-control study of breast cancer risk among Chinese women from Shanghai. They found that the valine allele was more common in cases than in control subjects (16% vs. 11%, respectively) with an odds ratio (OR) of 1.4 (95% confidence interval [CI] = 1.0-2.0). When they restricted the analysis to women aged 45 years and younger, the association with breast cancer remained (OR = 1.7; CI = 1.1–2.6).

Subsequently, 2 studies have specifically addressed the association with early onset of breast cancer observed by Xie et al.[24] A British hospital-based case-control study, with breast cancer cases restricted to diagnosis before the age of 40, found no association between the *HER2* valine allele and risk of breast cancer.[25] A population-based case-control study in Germany examined the relation of the *HER2* polymorphism with breast cancer risk in younger women (≤45 years), including the interaction with other known breast cancer risk factors.[26] Overall, the valine allele was not associated with breast cancer risk, but when stratified on a positive family history, there was a 2-fold increase in risk (OR = 2.1; CI = 1.0–4.8).

These reports prompted us to investigate whether the I655V *HER2* polymorphism was associated with breast cancer risk among a genetically distinct population using samples from the Washington community-based study of Ashkenazi Jews. This population allowed us to examine the effect of this polymorphism in details by age strata and family history to address the effect of this gene variant.[24-26] We further examined the possibility of the *HER2* I655V polymorphism as a genetic modifier of breast cancer risk in *BRCA1/2* carriers and in women with a family history of breast cancer.

MATERIALS AND METHODS

Study Subjects

We have previously described the details of recruitment of volunteer subjects from a Washington, DC Ashkenazi community-based survey, data collection, descriptive information about the subjects, and laboratory techniques.[3] Briefly, 5318 Jewish men and women over the age of 20 years provided a blood sample and a self-administered questionnaire that included detailed information on personal and first-degree relatives' cancer history. Results of *BRCA1/2* carrier status testing have been reported previously.[3] Mutation status was defined as the presence of 1 of the 3 founder mutations (185delAG or 5382insC in *BRCA1*, or 6174delT in *BRCA2*). An institutional review board of the National Institutes of Health approved this study. Subjects who gave specific permission for future anonymous use of their samples were included in the analysis.

HER2 *I655V Polymorphism Assay*

The primer sequences used for the I655V *HER2* polymorphism have previously been reported.[18] Details on the I655V assays can be accessed with the electronic version of this article at www.epidem.com.

Statistical Analysis

A large number of volunteers did not have a history of breast cancer in their female first-degree relatives and thus contributed little information on estimation of relative risk of breast cancer. We therefore selected an efficient subset of volunteers for *HER2* SNP analysis from the original 5082 volunteers who consented to the use of blood for future studies. The subset included all *BRCA1/2* mutation carriers (n = 120), all breast or ovarian cancer survivors, and all volunteers with a positive family history of breast cancer (n = 1217) plus 231 randomly chosen volunteers. For a population-specific *HER2* allele frequency estimate, we additionally tested 185 Ashkenazi subjects from an Israeli population panel that were obtained from the National Laboratory for the Genetics of Israeli Populations at Tel Aviv University, Israel.

Because volunteers with a personal and family history of breast cancer were oversampled for genotyping, the sample of selected volunteers and corresponding sample of relatives were not representative of the underlying population. All inferences from our study, however, were population-based in the sense that frequency and risk estimates were adjusted for in the sampling design so that they reflect the corresponding parameters in the underlying population. In particular, genotype frequency estimates based on the selected volunteers of the Washington, DC, study were adjusted for stratified sampling by a standard method of weighting each sampled subject with the inverse of the sampling fraction of the respective stratum.[27] Confidence intervals for the frequencies and the significance level for testing of the Hardy-Weinberg Equilibrium (HWE) were based on bootstrap resampling methods.[28]

For estimating age-specific absolute risk (penetrance) of breast cancer by *HER2* alone or by the joint status of *HER2* and *BRCA1/2*, we considered extensions of the kin-cohort approach. This method uses the reported cancer incidence among relatives of both the selected volunteers who were genotyped and the unselected volunteers who were not genotyped. Because this method uses cancer incidence data of all first-degree female relatives, it produces a valid estimate of absolute risk that is representative of the risk of the underlying population. We briefly describe the background of the kin-cohort method and the basic principles for the new extensions. Technical details are provided in

the Appendix with the electronic version of this article at www.epidem.com.

Kin-Cohort Estimation

Struewing et al.[3] estimated the age-specific cumulative risk (penetrance) of breast cancer from BRCA1/2 mutation using the breast cancer incidence data of first-degree relatives of a sample of genotyped volunteers (proband). Wacholder et al.[29] formally proposed the underlying analytic approach as the "kin-cohort" method. The method was developed to estimate the cumulative distribution functions $F_g(a)$ that gives the probability of disease onset at or before age a in a person with genotype g in the absence of competing causes of mortality. For an autosomal-dominant disease gene, the genotype g of an individual can be a represented as a binary indicator of whether the individual carries the mutation ($g = 1$) or not ($g = 0$). The cumulative risk of the disease among carriers, $F_1(a)$, is generally known as the penetrance function for the mutation.

In kin-cohort data, the genotypes of the relatives are typically not available and thus the cumulative risk functions $F_g(a)$, $g = 0,1$ cannot be directly estimated. The genotypes of the probands, however, can be used to predict the genotype distribution of the relatives. For rare mutations, Wacholder et al.[29] used Mendelian principles to show that slightly more than half of the first-degree relatives of mutation carriers are expected themselves to be carriers, whereas only a small fraction of first-degree relatives of the noncarriers are expected to be carriers. More precisely, they showed that for a rare mutation with allele frequency f, the odds of carrying the mutation for first-degree relatives of the mutation carrier is $(0.5 + f)$: $0.5 - f)$, whereas that for the first-degree relative of the mutation noncarrier is f:$(1 - f)$. Thus, they argued that the expected cumulative incidence functions for disease among the relatives of the carriers and among the relatives of the noncarriers are given by corresponding weighted averages of the cumulative risk function for the carriers and the cumulative risk function for the noncarriers. More precisely, the expected proportion of disease incidence up to or before age a among the relatives of carriers and the relatives of noncarriers are given by $(0.5 - f)F_0(a) + (0.5 + f)F_1(a)$ and $(1 - f)F_0(a) + fF_1(a)$, respectively. This gives rise to 2 equations in 2 unknowns which are then solved to obtain estimate of $F_0(a)$ and $F_1(a)$.

The concept of kin-cohort estimation of penetrance led to a series of methodologic investigations. A major limitation of the method-of-equations approach (or the method-of-moments approach, as it was later termed by Gail et al.[30]) is that it can produce nonmonotone estimate of cumulative risk function. Gail et al.,[30] Chatterjee and Wacholder,[31] and Moore et al.[32] have described various likelihood-based extensions of the kin-cohort estimation method that can overcome this and other limitations of the "method-of-moment" approach. Wacholder et al.[29] and Gail et al.[33] have described various practical advantages and disadvantages of the kin-cohort design in comparison with other population-based designs such as cohort or case-control studies. Gail et al.,[33] Wacholder and Chatterjee,[34] and Gail and Chatterjee[35] have described sources of biases in kin-cohort estimation. An article by Wacholder and Chatterjee[34] gives a comprehensive review of the current literature of the kin-cohort design.

We report here 2 extensions for kin-cohort estimation. First, we developed a method of kin-cohort estimation that can use family history of the unselected volunteers who were not genotyped for *HER2* I655V variation. In ordinary kin-cohort estimation, we assume that there are 2 groups of subjects, the relatives of the carriers and the relatives of the noncarriers, who have 2 separate genotype distributions. In the new extension, we explicitly consider a third genotype distribution that applies to relatives of volunteers who have not been genotyped. In particular, because nothing is known about the genotype of the third group of relatives, we propose to estimate their genotype distribution in an unbiased manner by applying the corresponding population distribution of the genotypes. In the electronic version of this article at www.epidem.com, we show how to combine the disease history data of these 3 groups of relatives to form a "marginal likelihood," which in turn can be maximized to obtain an estimate of penetrance.

We developed a second extension of the kin-cohort method to estimate penetrance of breast cancer by joint mutation status of *BRCA1/2* and *HER2*. The same principle of original kin-cohort estimation applies here. The relatives of the volunteers who are genotyped for both genes are classified into the following 4 groups: relatives of *HER2-/BRCA1/2-*, relatives of *HER2+/BRCA1/2-*, relatives of *HER2-/BRCA1/2+*, and relatives of *HER2+/BRCA1/2+*, where + or - indicates presence or absence of the variation/mutation. For each of these 4 groups of relatives, a distribution of joint genotype status can be predicted based on Mendelian principle. We assumed that the 2 genes are unlinked and computed the joint genotype distribution of the relatives, given that of the volunteers, as the product of the individual conditional (transmission) probabilities. The relatives of the volunteers who are genotyped only for *BRCA1/2* can be classified into the following 2 groups: relatives of *BRCA1/2-* and relatives of *BRCA1/2+*. For each of these types of relatives, we calculated the joint genotype distribution as the product of the conditional distribution for *BRCA1/2* given the volunteers' *BRCA1/2* status and the population distribution for *HER2*. Based on the various genotype distributions, we then computed the expected cumulative disease incidence rate for each group of relatives in terms of a weighted average of the underlying cumulative risks of the disease corresponding to the following genotypes: *HER2-/BRCA-*, *HER2+/BRCA-*, *HER+/BRCA-*, and *HER2+/BRCA+*. Finally, we obtained

the estimate of these cumulative risk functions by maximizing an appropriate "marginal likelihood" of the data that combines the disease incidence data of the various types of relatives.

RESULTS

We analyzed 1375 samples from the Washington, DC volunteers (193 did not amplify), as well as 185 subjects from the reference Israeli Ashkenazi population panel, for the *HER2* polymorphism. We found genotype frequencies of 76.1% (CI = 73.5–78.7) Ile/Ile, 22.4% (CI = 19.9–24.9) Ile/Val, 1.5% (CI = 0.9–2.2) Val/Val, 73% (70.1–75.9) Ile/Ile, 24% (CI = 21.3–26.6) Ile/Val, and 3% (CI = 2.6–3.4) Val/Val. The genotype frequency estimate from WAS did not deviate from Hardy-Weinberg equilibrium ($P = 0.35$).

The Kaplan-Meier breast cancer incidence curves began to diverge at age 40, with cumulative incidence of the relatives of valine carriers remaining consistently higher than that of the relatives of noncarriers up to age 70 (Fig. 1A). The corresponding kin-cohort estimates for the age-specific cumulative risk curves for the carriers and the noncarriers showed the same pattern (Fig. 1B; Table 1). In the relative-risk scale, however, there is clearer separation because the carriers and the noncarriers are compared directly. We note that the cumulative incidence curves in Figure 1A reflect the risk of the disease in the relatives of the selected volunteers who were genotyped. By design, the sample of selected volunteers were enriched by subjects who had a family history of breast cancer, and therefore the cumulative incidence curves in Figure 1A are not representative of the absolute risk of the disease in the underlying population. The kin-cohort estimates (Fig. 1B), however, account for family history of the disease for all volunteers in the study and thus are representative of the absolute risk of the disease in the underlying population.

Overall, the lifetime risk of breast cancer (cumulative risk until age 70) was approximately 30% higher (RR = 1.33; CI = 1.03–1.83) among carriers of valine allele than among noncarriers. This elevation in risk was more prominent at young ages; the cumulative risk until age 50 was twice as high in carriers as in noncarriers (RR = 2.11; CI = 1.39–3.28). Inspection of the age-specific hazard estimates (interval risk; Table 2) suggested the strongest effect of *HER2* valine allele for the age intervals 40-49 (HR = 2.25; CI = 1.34–3.60). The hazard estimates also suggested that the valine allele did not have an effect on risk of breast cancer in women older than 60. The homozygous and heterozygous carriers, when treated separately (not shown), showed no difference in risk.

By restricting the kin-cohort analysis to the relatives of the volunteers who had a personal history of breast cancer, we obtained an estimate of breast cancer risk associated with the valine allele among women who had at least 1 affected relative. Although for the relatives of noncarrier cases there were 57 of 503 incidences of breast cancer, there were 28 of 128 incidences of the disease in the relatives of carrier cases. The kin-cohort estimates of age-specific relative risks (Table 3) showed an age-dependent pattern similar to that reported in Table 1. The magnitude of the relative risks, however, was larger. The strongest effect of the valine allele was again seen in the age group younger than 50 who had a relative risk of 8.9 (CI = 1.9–19.7).

The age-dependent effect of *HER2* I655V had a similar pattern when evaluated for *BRCA1/2*-positive and -negative subjects separately (Fig. 2). For both carriers and noncarriers of *BRCA1/2* mutations, the valine allele seems to be associ-

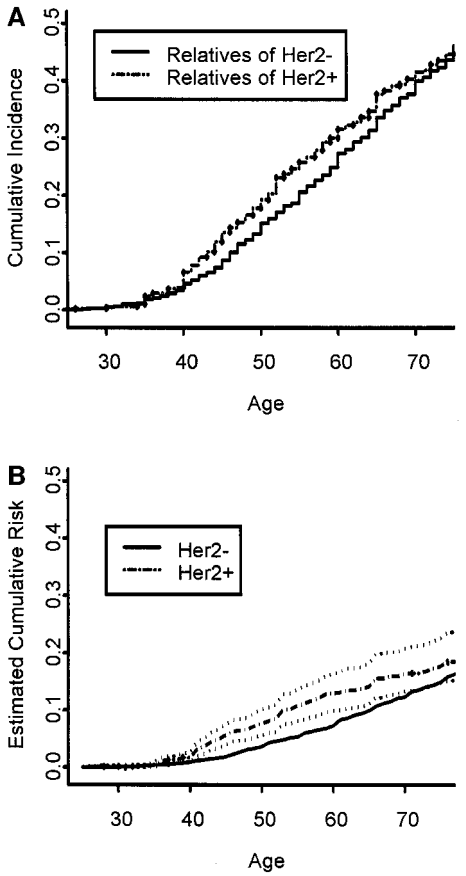

FIGURE 1. Cumulative risk estimation of the I655V polymorphism in Washington, DC relatives. (A) Observed breast cancer incidence of the relatives of volunteers who were genotyped for Her2. The number of Her2- (valine noncarriers) women at risk for breast cancer by age 40 was 1808; by age 70 it was 625. The number of Her+ (valine carriers) women at risk by age 40 was 564; by age 70 it was 194. B. Kin-cohort estimation of breast cancer risk in valine allele carriers (+) and noncarriers (-). Dotted lines represent 95% confidence intervals for the carriers.

TABLE 1. Cumulative Risk of Breast Cancer in HER2 Valine Allele Carriers and Noncarriers by Age (years)

Age	Ile/Ile Risk (CI)	Ile/Val, Val/Val Risk (CI)	Cumulative Risk Ratio (CI)
<40	0.010 (0.007–0.012)	0.017 (0.010–0.026)	1.78 (0.82–3.58)
<50	0.037 (0.030–0.044)	0.078 (0.056–0.102)	2.11 (1.39–3.28)
<60	0.073 (0.063–0.083)	0.129 (0.099–0.163)	1.76 (1.25–2.43)
<70	0.123 (0.109–0.133)	0.164 (0.133–0.208)	1.33 (1.03–1.83)

TABLE 2. Breast Cancer Hazard in HER2 Valine Carriers and Noncarriers by Age (years)

Age	Ile/Ile Hazard (CI)	Ile/Val, Val/Val Hazard (CI)	Hazard Ratio (CI)
<40	0.010 (0.007–0.012)	0.017 (0.010–0.026)	1.78 (0.82–3.58)
40–49	0.027 (0.022–0.034)	0.061 (0.043–0.083)	2.25 (1.34–3.60)
50–59	0.038 (0.031–0.045)	0.057 (0.036–0.083)	1.48 (0.85–2.48)
60–69	0.054 (0.043–0.061)	0.040 (0.020–0.069)	0.74 (0.33–1.47)

TABLE 3. Cumulative Risk of Breast Cancer by Age Among HER2 Valine Carrier and Noncarrier Women Who Reported a Positive Family History of Breast Cancer

Age	Ile/Ile Risk (CI)	Ile/Val, Val/Val Risk (CI)	Cumulative Risk Ratio (CI)
<40	0.008 (0.000–0.021)	0.022 (0.000–0.050)	2.71 (NA)*
<50	0.019 (0.009–0.048)	0.170 (0.068–0.225)	8.93 (1.91–19.74)
<60	0.055 (0.032–0.101)	0.236 (0.116–0.301)	4.29 (1.55–7.77)
<70	0.122 (0.082–0.180)	0.284 (0.155–0.378)	2.34 (0.97–4.05)

*CI for hazard ratio <40 years was not obtained as a result of small numbers.

ated with elevated risk. However, these results are based on small numbers.

DISCUSSION

We report a modest association between the *HER2* valine polymorphism and lifetime risk of breast cancer with a stronger effect of the allele at early ages and in women with a positive family history of breast cancer. For the overall effect of the valine allele on lifetime risk, (cumulative risk until 70), the relative risk was 1.33, with the confidence intervals excluding 1.0. It is worth noting that our relative lifetime-risk estimate is very close to the overall relative risk estimate of Xie et al.[24] who reported an odds ratio of 1.4 (CI = 1.0–2.0). Similar to Xie et al., we also found the association to be stronger in younger women, although 2 other studies have found no elevation of risk of breast cancer associated with the valine allele among young women (<45 years).[25,26] Our analysis also suggests that the valine allele might not have any effect among women older than 60. This raises the possibility that the risk associated with carrying the *HER2* valine allele might predominantly affect pre- or perimenopausal breast cancer.

In our study, volunteers with a personal and family history of breast cancer were oversampled for testing of the *HER2* variation. To account for the stratified sampling design, we developed an extension of the kin-cohort method that can incorporate reported cancer incidence in relatives of both tested and nontested volunteers. For comparison purposes, we also evaluated the kin-cohort results restricted to the 231 subjects who were truly randomly selected for testing of the *HER2* variation. The resulting estimate and shape of the cumulative risk functions for carriers and noncarriers, within limits of uncertainty, were comparable to those we reported in Figure 1. In particular, this analysis estimated the relative lifetime risk associated with *HER2* to be 1.68 (CI = 0.82–3.01).

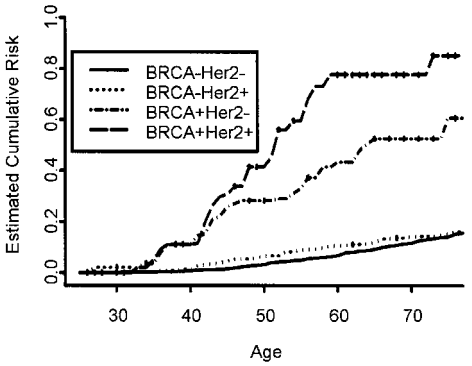

FIGURE 2. Kin-cohort estimation of breast cancer by joint HER2 and BRCA1/2 status in Washington, DC relatives.

The kin-cohort method, when applied only to the relatives of the breast cancer cases, allowed unbiased comparison of risk in the valine allele carriers and noncarriers who reported a relative with breast cancer. This analysis suggests that the effect of the valine allele might be stronger among women with a family history of the disease, confirming previous findings.[26] A stronger effect of the valine allele among women with a positive family history suggests the possibility of gene-gene interaction. Our analysis, however, does not provide evidence for an interaction between the *HER2* polymorphism and the 2 most penetrant breast cancer genes identified to date, *BRCA1* and *BRCA2* (Fig. 2 and data not shown).

These findings contribute to the recently published literature[24-26]and suggest that the *HER2* I655V allele is either a susceptibility allele of breast cancer or a marker in linkage with another allele. Our large sample size and the retrospective follow-up data of the relatives allowed us to examine the age-related effect in greater detail than has been reported before.[24-26] One potential problem in studying the effects of the germline *HER2* polymorphism on breast cancer risk is that if survival times after diagnosis are diminished in the polymorphism carriers (like in women whose breast tumors over-express Her2), then analysis on prevalent cases would likely underestimate the valine allele frequency. However, we avoid this possible bias by using the kin of the volunteers rather than the volunteers themselves to estimate cancer risk. This is a known strength of the kin-cohort method.[29,31,33] In doing so, survival time after breast cancer diagnosis in relatives is irrelevant.

A possible weakness of this study is that the absolute levels of breast cancer risk might be overestimated because the volunteers who participated in the original study were not a random sample of the population. Approximately twice as many volunteers as expected reported a close family history of breast cancer, and this would tend to bias our estimates upward. However, our main interest was the relative risk associated with the *HER2* I655V SNP and *BRCA1/2* genotypes, and the kin-cohort method should be a valid means of evaluating this, providing volunteers do not participate in the study based on knowledge of their genotype status.[29] We observed an elevated risk associated with the 655-valine allele. The magnitude of the risk was similar in women who were not predicted to be carriers of a *BRCA1/2* mutation and also in the much smaller group of women who were *BRCA1/2* mutation-positive.

Results on the association of genetic polymorphisms with breast cancer are inconsistent across studies,[36] and there is not enough evidence to justify the use of any single polymorphic variant for risk identification in a clinical setting. However, the clinical implications of breast cancers in relation to Her2 over-expression are well documented.[14,16,37,38] Our results provide a foundation for future studies to examine the prevalence of the *HER2* valine allele in women with Her2 over-expressing breast cancers, which is of potential clinical interest because women with breast cancers are now routinely screened for Her2 expression.

Our results suggest an age-specific increased risk of breast cancer that is magnified with family history. These results give impetus to future study of the biochemical consequences of the I655V polymorphism, as well as to testing whether the *HER2* I655V polymorphism influences risk of somatic over-expression or amplification of Her2. Various aspects of this polymorphism, such as its effect on dimerization with other Her receptor partners, ligand binding, signal transduction, and receptor stabilization/degradation, can be directly measured to determine how the receptor kinetics are influenced by the *HER2* polymorphism and possibly by menopausal status and hormonal regulation.

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